Amoebic and Giardia Infections among Diarrhoeal patients in Lideta Health Center, Addis Ababa, Ethiopia

By
Abiyot Getachew

Supervisor: Dr. Haileeyesus Adamu,
Assoc. Professor, Institute of Biotechnology

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List of Acronyms

AIDS	Acquired Immunodeficiency Syndrome
CDC	Center for Disease Control and Prevention
CSA	Central Statistical Agency
DNA	Deoxyribonucleic Acid
EPHI	Ethiopian Public Health Institute
PCR	Polymerase Chain Reaction
PVA	Poly Vinyl Alcohol
RPM	Rotations Per Minute
SPSS	Statistical Package for Social Science
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organization

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Abstract

Amoebic and giardia infections *are one of the major public health problems in many countries including Ethiopia. They are more common in children due to poor personal hygiene and lack of awareness. The objective of the present study was to identify intestinal protozoan parasite species and to determine their prevalence among patients visiting Lideta health center, Addis Ababa, Ethiopia. The design of the study was a cross-sectional parasitological survey involving examination of fresh stool drawn from patients visiting the health center, during September 2017- May 2018. Data were gathered by means of questionnaire survey and laboratory parasitological examination procedures. The stool samples were examined using direct wet-mount and formol-ether concentration methods. From the total of 240 study participants, 122(50.8%) were males and 118(49.2%) females. In the study 27(22.1%), 17(13.9%) males and 23(19.5%), 20(16.6%) females were positive for Entamoeba histolytica /dispar/ moshkoviskii and Giardia lamblia respectively. The overall prevalence of amoebic and giardia infections was 36.3 %. The prevalence of amoebic and giardia infections was significantly associated with some risk factors such as habit of eating uncooked vegetables and unwashed fruits (p= 0.014), unhygienic toilet (p= 0.042), hand washing before meal or after toilet (p= 0.001) and cleanliness of kitchen utensils (p= 0.017). In general, the study revealed that IPIs represented a major public health problem in terms of morbidity and economic consequences. It is therefore recommended to local (woreda) health sector and any concerned bodies that systematic, integrated and community-participatory IPIs, prevention and control programs need to be implemented in the study area.*

Key-words: *Health center, Amoebic and Giardia infections, Lideta, Prevalence*

1. Introduction

1.1. Back ground

Protozoan parasitic infections are among the most common intestinal parasitic infections worldwide and the same is true in Ethiopia. High prevalence is found in people with low socio-economic status, poor living condition, overcrowded area, poor environmental sanitation, improper garbage disposal, unsafe water supply and unhygienic personal habits. Diarrhoea is the major symptom shown by the protozoan parasitic infected patients. Intestinal parasitic infections are still an important public health problem. Human beings have been exposed to diverse groups of intestinal protozoan parasites. Over 60 species of protozoan parasites cause diseases on people worldwide. *Entamoeba histolytica* and *Giardia lamblia* are estimated to infect about 600 million and 200 million people worldwide, respectively (Murray *et al*., 2002).

Human intestinal protozoan parasites are identified as cause of morbidity and mortality throughout the world particularly in developing countries including Ethiopia. There is more prevalence throughout the tropics, especially among poor communities. A high prevalence of intestinal protozoan parasitic infections in human is positively correlated with poverty and poor personal hygiene, lack of safe water supply and contamination of the environment by human excreta and animal wastes. Intestinal protozoan parasitic infections increase host's susceptibility to other infections and diminish learning ability and growth especially in growing children (Karaman *et al*., 2006). Intestinal protozoan parasite species are responsible for some of the most devastating and prevalent diseases of human. Intestinal protozoan parasitic infections constitute a global health burden causing clinical morbidity in 450 million people; many of these were women of reproductive age and children in developing countries (Quihui, 2006).

Enteric protozoa are uni-cellular microscopic parasites inhabiting the gastrointestinal tract of numerous vertebrate hosts. Of these protozoa, *Giardia lamblia*, *Entamoeba histolytica* and Cryptosporidium spp. are considered responsible for the majority of human infections (Kosec *et al.,* 2001, and Haque *et al*., 2003). Human infections caused by *Giardia lamblia* and Cryptosporidium spp, are termed giardiasis and cryptosporidiosis respectively. Although the greatest burden of both diseases occurs in developing countries, Cryptosporidium has been recognized as a major cause of many water-borne and food-borne outbreaks of gastroenteritis in developed countries (Nichols, 2000). *Giardia lamblia* is also considered as the main cause of non-viral non-bacterial diarrhea in developed countries (Hoque *et al.,* 2002). Similarly, human disease caused by *E. histolytica* is named Amoebiasis. Infection with *E. histolytica* has been reported in many countries but the highest prevalence rates are reported in developing countries. Due to the invasive potential

of *E. histolytica*, approximately 100,000 cases from a global burden of 50 million cases are thought to result in death each year (Stanley, 2003).

Parasitic diseases are incriminated in causing more than 33% of global deaths of which intestinal parasitic infections are believed to take the major share (WHO, 2004). Lack of safe drinking water and environmental sanitation are largely responsible for more than 800 million expected cases of diarrhoeal diseases and 4.5 million associated deaths in many developing countries every year. Morbidity and mortality due to diarrhoeal diseases in developing countries remains to be the main public health problems that need attention. Although there could be many other causes of diarrhea, the enteric protozoa Cryptosporidium parvum and *Giardia lamblia* have been recognized as important causes of both outbreak related sporadic diarrhoea in humans of immune competent and immune compromised individuals could be the victims of diarrhoeal diseases due to these parasites (Mbae *et al*., 2013).

Reports from different parts of Ethiopia shown that there was different prevalence rate of Amoebiasis, Giardiasis and Cryptosporidiosis (Amare *et al*., 2007 and Ayele, 2006). The prevalence of Cryptosporidium infection in children with diarrhea ranged from 3.3% in Jimma, 5.6% in Addis Ababa to 9% in North Western Ethiopia. A number of survey and routine diagnosis in Ethiopia indicate that Amoebiasis is one of the most widely distributed intestinal diseases (Gebru and Girma 2000).

Epidemiological surveys have indicated that the most important source for human parasitic infection is contaminated and recreational water, food, household animals, improper garbage disposal and poor environmental sanitation. Infected people in our country with high prevalence of intestinal protozoan infection are attributable to factors associated with low socio economic status. Such factors include poor personal hygiene, environmental sanitation, low income, and low level of education, improper sanitation of dining utensil, toilet facility and lack of clean water supplies. For instance Ethiopia has one of the lowest quality drinking water supply and latrine coverage (Mengistu *et al*., 2007).

Therefore, the aim of this study was to assess intestinal protozoan infections among diarrhoeal patients and associated factors in Lideta health center.

1.2. Statement of the problem

The relationship between epidemiological factors and prevalence of amoebic and giardia infections in the community has not been fully investigated in Lideta health center and the surrounding area, and organized control programs targeting transmission factors are lacking. Transmission depends on sanitation related issues and the number of the asymptomatic carriers.

Lideta sub-city particularly the study area has poor sewage disposal system and absence of toilet facilities. House without sewage system increased the risk for diarrhoea in children (Shrestha *et al.,* 2012). Epidemiological surveys indicated that high prevalence of amoebic and giardia infections are attributable to factors associated with low socio-economic status including poor personal hygiene, environmental sanitation, low household income, low level of education, and improper sanitation of kitchen utensils, resident areas of parents, toilet facility and lack of clean water supplies. For instance, Ethiopia has one of the lowest quality drinking water supply and latrine coverage (Mengistu *et al*., 2007).

Even though, several studies have been conducted on the prevalence of intestinal protozoan infections in Ethiopia, there are still several localities in the country including the study area, Lideta health center, in which epidemiological information about the prevalence of amoebic and giardia infections among patients is not available. Therefore, the purpose of this study was to obtain information about prevalence or distribution of amoebic and giardia infections among patients and associated factors in Lideta health center.

1.3. Significance of the study

New efforts are being made to improve understanding of the epidemiology of sanitation related amoebic and giardia infections and intensify control efforts against these parasites. To contribute to a better comprehension of the epidemiology of the *E. histolytica* and *G. lambilia,* their prevalence or distribution and their association with risk factors was determined among patients in Lideta health center. The description of the relationship between the major risk factors with infections with *E. histolytica* and *G. lamblia* will contribute to the development of an integrated treatment and control programs for these parasites in the study area.

Therefore, the present study was conducted to determine the prevalence of amoebic and giardia infections and the associated risk factors in patients visiting Lideta Health Center which is a prerequisite to formulate the appropriate control systems like health education and minimize the risk of infection by raising the awareness of the people on the transmission, prevention and mode of infection of the disease.

2. Literature Review

2.1. Human Intestinal Protozoan Parasitic Infection

Protozoa are a diverse group of organisms that have evolved to occupy a variety of ecological niches. There are over 30 phyla of protozoa; Most of these have evolved a totally parasitic existence. The enteric protozoa that cause human illness are usually transmitted by the consumption of food and drink, or through environmental contamination and poor hygiene. Gastrointestinal illness created towards pathogenic protozoa is related to an elevated degree of morbidity and mortality worldwide, particularly in children. In developing nations, protozoan infections were registered every year in 58 million children (Calderaro *et al.,* 2014).

Intestinal protozoan diseases are caused by unicellular microorganisms which invade the wall of the intestine such as Amoebiasis, Giardiasis, and Cryptosporidiosis. Numerous protozoa inhabit the gastro-intestinal tract of humans. The majority of intestinal protozoa is non-pathogenic commensals, or only result in mild disease. Some of these organisms can cause severe disease under certain circumstances. Apicomplexa and microsporidia species, which normally do not evoke severe disease, can cause severe and life-threatening diarrhea in AIDS patients and other immunocompromised individuals (Adamu *et al.,* 2006).

Intestinal protozoan parasite infections are a significant problem with more than 58 million cases in children each year. Pathogenic intestinal protozoa are especially important in the developing world where they may cause death. Most intestinal protozoan parasites are spread by faecal–oral contact or contamination of water or food. Poor sanitation and poverty are contributory factors in many low income countries. Symptoms of intestinal protozoan parasite infections include diarrhea, abdominal pain, nausea, vomiting and weight loss (Ngonjo, *et al.,* 2012).

2.2. Life Cycle of Intestinal Protozoan Parasites (*E. histolytica/dispar* and *G. lamblia*)

Several members of the genus *Entamoeba* infect humans. Among these only *E. histolytica* is considered pathogenic and the disease it causes is called Amoebiasis or amebic dysentery. *E. dispar* is morphologically identical to *E. histolytica,* but is not pathogenic. The two species are found throughout the world, but like many other intestinal protozoa, they are more common in tropical countries or other areas with poor sanitary conditions. It is estimated that up to 10% of the world Population may be infected with either *E. histolytica* or *E. dispar* and in many tropical countries the prevalence may approach 50%. It is also estimated that about 100,000 deaths and 50 million cases of Amoebiasis occur per year in the world and humans are the only host of *E. histolytica* and there are no animal reservoirs (Haque *et al.,* 2003).

E. histolytica is reported to be the most prevalent (65.5%) among Burkina Faso school children (Erismann *et al.,* 2016). Thus it is ranked second next to malaria as the cause of mortality due to this intestinal protozoan parasite infection. The parasite normally inhabits the large intestine but is also capable of invading other organs such as the liver, brain and spleen. The majority of amoebic infections are reported to occur in Central America, South America, Africa and Asia. These are often associated with poor water and food hygiene, and sanitation practices.

The life cycle of *Entamoeba histolytica* as shown in figure-1 includes the infective cyst and the invasive trophozoite forms. Infection is acquired by ingestion of infectious cyst through water or undercooked food contaminated by human faeces. After ingestion of the cyst, which is resistant to gastric acids and enzymes, excystation occurs in the ileum, part of the small intestine, to form trophozoites. The trophozoites are larger in size and actively motile organisms. According to the bind-lyse-eat model, the trophozoites bind to the large intestine and invade the wall releasing amoeba pores and phospholipases, causing ulceration of the mucous membrane (called flask shaped ulcers), and sometimes large vessels may be eroded and severe intestinal hemorrhage result (Petri and Singh, 2006).

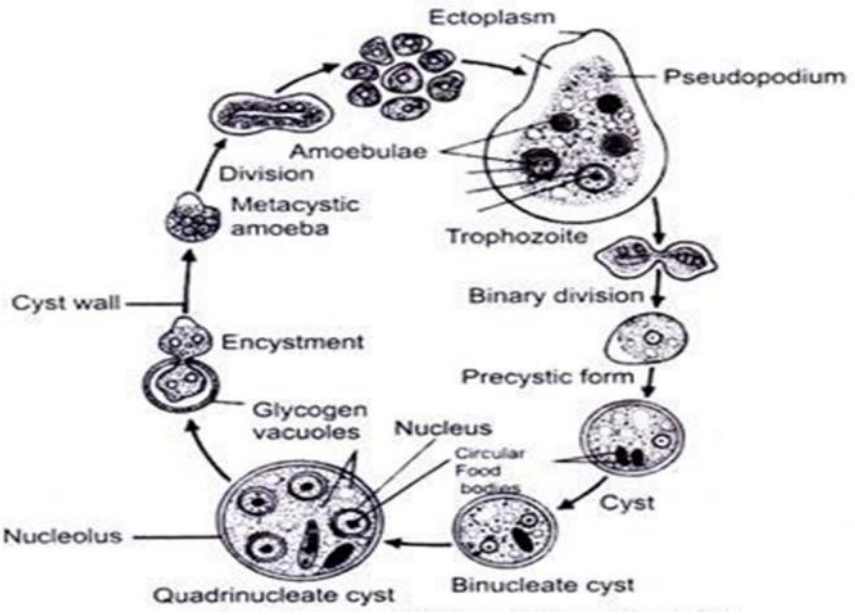


Figure 1: Life cycle of *Entamoeba histolytica*
Source: http://www. dpd.cdc.gov/dpdx

The parasite *Giardia lamblia* reproduces by binary fission which is a type of reproduction in which one cell divides into two new cells by mitosis. During the growth cycle the components of the *Giardia* cell multiply so that each daughter cell would be a complete copy of the original parent cell. The newly formed cells then pinch off from each other; in so doing a complete reproduction cycle would occur the infective stage of *G. lamblia*, the cyst, is elliptical in shape and its size ranges from 6 to 10 microns and contains two to four nuclei. The cyst possesses a structure that enables it to be resistant to most environmental factors and disinfection and make it successful in being the infective stage of the parasite. The cyst has a thin and protective wall that allows it to survive in feces for weeks or for about 3 months in cold water (Monib *et al.*, 2016).

Giardiasis could be contracted through drinking contaminated waters or ingestion of contaminated food stuffs. The cyst passes through the stomach and enters the small intestine. The acidic environment of the stomach could not harm the cyst because it has a thin protective wall to protect it until it reaches the alkaline environment, the small intestine. This alkaline environment initiates excitement of the cyst. During excystation, the cyst wall raptures at the pole opposite to the nuclei so that the flagella and other projections emerge from the rupture point. The cyst wall is then completely shed and the parasite will enter into its trophozoite stage (Kinuthia *et al.*, 2014).

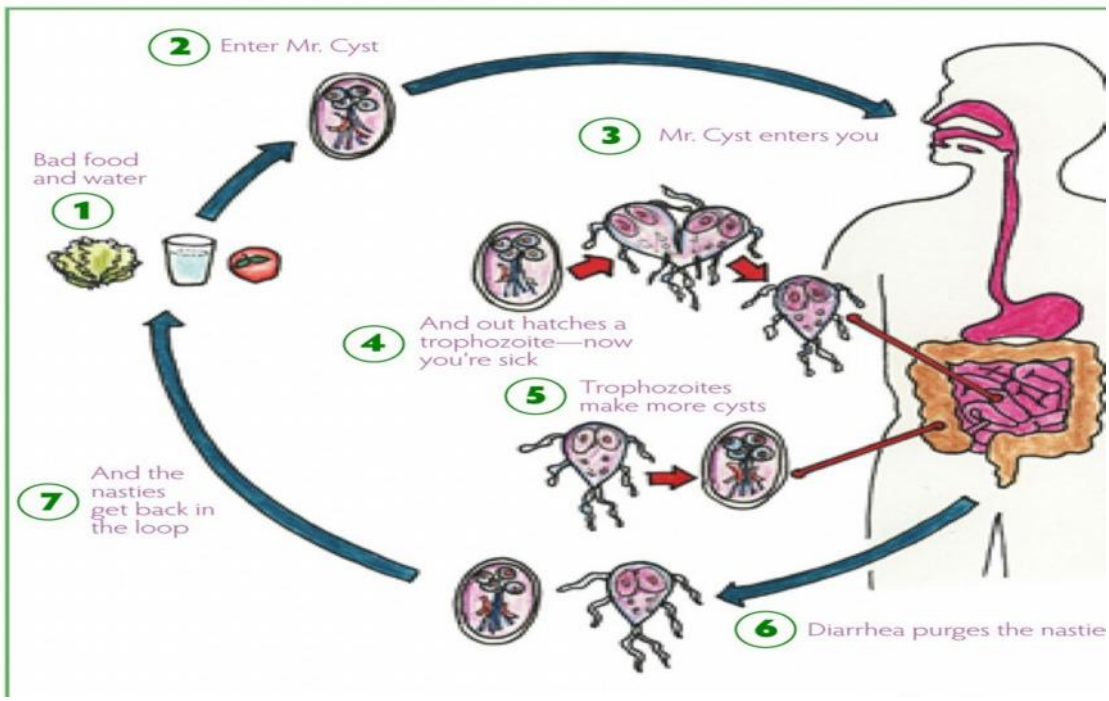


Figure 1 : Life cycle of *Giardia lamblia*
Source: http://www.dpd.cdc.gov/dpdx

2.3. Pathogenesis and Clinical Manifestation

Intestinal protozoan parasitic infection can result in gastrointestinal disease in humans. As a result of infection of the parasite more or less similar clinical sign and symptom can be observed. For example Infections with *E. histolytica/dispar* have no symptoms in many individuals, and most can clear their infection without any signs of disease. For unexplainable reason, however, 4-10 % of asymptomatic individuals infected with *E. histolytica/dispar* develop disease over a year. In other words, different studies indicate that up to 90 % of *E. histolytica* infections, the symptoms are absent or very mild .There is a wide spectrum of clinical presentations of *E. histolytica* infection Symptomatic Amoebiasis is primarily an intestinal disease, and when it becomes extra intestinal, it usually involves the liver. Pathogenesis of amebiasis is believed to be a multi-step, multifactorial process. Though a large number of studies have attempted to unravel the factors/molecules responsible for the pathogenesis of Amoebiasis, the processes involved in pathogenesis are poorly understood. The aspects of pathogenesis which have been investigated experimentally can be broadly categorized into mechanisms involving (i) interactions with the intestinal flora, (ii) lysis of target cell by direct adherence, (iii) lysis of target cell by release of toxins and (iv) phagocytosis of target cells (Butel *et al.*, 2007).

Symptoms of Amoebiasis could be acute (Frequent dysentery with necrotic mucosa and abdominal pain) and chronic (Recurrent episodes of dysentery with blood and mucus in the feces). There are intervening gastrointestinal disturbances and constipation. Cysts are found in the stool. The organism may invade the liver, lung and brain where it produces abscesses that result in liver dysfunction, pneumonitis, and encephalitis (WHO, 2002).*G.lamblia* is usually weakly pathogenic for humans. Cysts may be found in large numbers in the stools of entirely asymptomatic persons. In some persons, however, large numbers of parasites attached to the bowel wall may cause irritation and low-grade inflammation of the duodenal or jejunal mucosa, with consequent acute or chronic diarrhea associated with crypt hypertrophy, villous atrophy or flattening, and epithelial cell damage. The stools may be watery, semisolid, greasy, bulky, and foul-smelling at various times during the course of the infection. Malaise, weakness, weight loss, abdominal cramps, distention, and flatulence can be occurred. Children are more liable to clinical Giardiasis than adults. Immunosuppressed individuals are especially liable to massive infection with severe clinical manifestations. Symptoms may continue for long periods (Butel and Stephen, 2007).

As in any parasitic infections, host parasite interaction is the initial steps in the pathogenesis of giardiasis. In this interaction, first the *Giardia* trophozoites attach to the cell surface of villi by means of a disk on their posterior or ventral surface. Lectin, a protein on the trophozoite lining, recognizes specific receptors on the intestinal cell and

may be partly responsible for the tight attachment between the parasite and the villi following attachment of trophozoites, there will be major structural and functional abnormalities in the small intestine. Some of these abnormalities include mucosal damage as a result of mechanical obstruction or blockage of the intestine by a large number of parasites, the release of cytopathic substances such as thiol proteinases water intended for consumption, thoroughly washing hands before handling food, maintaining good personal cleanliness, properly disposing of fecal material and information dissemination through print media to educate the public regarding the dangers of giardiasis (Backer, 2000).

2.4. Epidemiology and Transmission

2.4.1. Global epidemiology of intestinal protozoan parasitic infections

Intestinal protozoan parasitic infections enjoy a wide global distribution. They are estimated to affect 3.5 billion people most of who are children and young residing in developing countries. The major intestinal Protozoan species of global public health concern are: *Entamoeba histolytica, Giardia lamblia and Cryptosporidium* species (WHO, 2000). The majority of infections are associated with poverty such as reduce access to safe drinking water, housing and inadequate access to health care. They also are affected by poor family and community hygiene and sanitation practices and prevailing climatic and environmental conditions (Jemeneh, 2001). These conditions lay stage for the continuous transmission of the Intestinal protozoan infections.

Intestinal protozoan infections are endemic worldwide and have been described as constituting the greatest single worldwide cause of illness and disease. Poverty, illiteracy, poor hygiene, lack of access to potable water and hot and humid tropical climate are the factors associated with intestinal parasitic infections. Parasitic Intestinal protozoa and helminthes are responsible for some of the most devastating and prevalent diseases of humans. Intestinal protozoan infections (IPIs) constitute a global health burden causing clinical morbidity in 450 million people, many of these women of reproductive age and children in developing countries (Quihui, *et al*.,2006).

Intestinal protozoan infections are among the most common infections in the world and are responsible for considerable morbidity and mortality (Kongs *et al.,* 2001). The epidemiology of intestinal protozoan parasitic infections shows that these parasites are found in every age group and in both sexes. However, the incidence is high in some areas and in some age groups. Human intestinal parasitic infections have a worldwide distribution, with the greatest incidence and intensity occurring in developing countries (Mccarthy *et al.,* 2004). Invasive Amoebiasis is prevalent in certain areas of the world including West and South-east Africa, China, and Mexico. The high occurrence of these

parasites is often related to poverty, poor living conditions and hygiene, and inadequate sanitation and water supply. In Turkey it was noted that the prevalence of pathogenic parasites was high among people who had no toilets in their houses. In Tehran Province, the highest infection rate (41.5%) was related to protozoan parasites, *E. histolytica* has been recovered worldwide and is more prevalent in the tropics and sub-tropics than in colder climates. However, in poor sanitary conditions in temperate and colder climates, infection rates have been found to equal that seen in the tropics. In a related study in Ardabil Iran, a total of 10 species were identified with *Giardia lamblia* (14%), *Blastocystis hominis* (10%) and *Entamoeba coli* (4.1%) being the most common parasites (Aksoy *et al.,* 2005).

Giardia lamblia also has a worldwide distribution with an incidence rate of between 11% and 30%. In the United States of America, it is now considered to be the most common intestinal parasite of man and the leading cause of diarrhoea due to protozoan infections in humans It is also the most frequently reported intestinal parasite in Peru (Beltran *et al.*,2004). Intestinal infections in general affect more than two-thirds of the human population and mostly children .The intensity of infection is a major determinant of morbidity and approximately reflected in the number of characteristic cysts passed out in faeces. Giardiasis is one of the most common parasitic infections having a worldwide distribution and occurring both in developed &developing nations. In Africa, Asia and Latin America about 200 million cases have been estimated to occur annually. In Ethiopia surveys across all regions of the country show giardiasis prevalence to be around 10% in the 1970s and early 1980s and it is more common in children than in adults. Cryptosporidium is known to cause diarrheal diseases in immunocompetent people and shown to be especially common among persons with AIDS or other forms of immune deficiency. The application of PCR assays to identify *Cryptosporidium* species from stool samples has shown that *C. hominis* and *C. parvum* are the major causes of human cryptosporidiosis (Cacciò, 2005). Interestingly, the prevalence of these species varies in different regions of the world.

2.4.2. Epidemiology of intestinal protozoan parasitic infections in Ethiopia

According to 1996 Federal Ministry of Health of Ethiopia reported that more than half a million out patients visited hospital/clinic due to intestinal protozoan parasitic infections. However, this might be an underestimate, as most of the health institutions lack appropriate diagnostic methods to detect parasites with small detection limits. In addition, some of the diagnostic methods for specific intestinal parasites, especially for the newly emerging opportunistic intestinal parasites, were not available to most health institutions. Among the common intestinal protozoan parasites *G. lamblia* and *E. histolytica/dispar* are widely distributed in Ethiopia. *Cryptosporidium* is now becoming a common opportunistic intestinal parasite in Ethiopia even though it is not diagnosed

routinely. Reports from different parts of the country showed different prevalence rates of cryptosporidiosis. Recently a study conducted in Lege Dini, rural area in Dire-Dawa, shown the prevalence of cryptosporidiosis to be 12.2 % (Ayalew *et al.,* 2008).

Another report indicated that the prevalence of *Cryptosporidium* among diarrhoeal patients referred to Ethiopian Public Health Institute (EPHI) was 20.6 % (Endeshaw *et al.,* 2004). The prevalence of *Cryptosporidium* infection alone in children with diarrhoea ranged from 3.3 % in Wukro Town, 5.6 % in Addis Ababa, and 9 % in North-western Ethiopia (Mersha and Eleni *et al.,* 2014 and Atnafu *et al.,* 2010). Another study with special emphasis on opportunistic parasitic infections among paediatric diarrhoeal patients in visiting hospitals in Addis Ababa, showed that the rate of *Cryptosporidium* spp. infection among these patients to be 8.1% (Gebru and Girma, 2000).

2.4.3. Factors affecting epidemiology and transmission of human intestinal protozoan parasitic infection

Each environmental change of natural phenomena or through human intervention alters the ecological balance. Deforestation and ensuring changes in land use, human settlement, commercial development, construction of roads, water control systems (dams, canal, and irrigation system) and climate change have been accompanied by global increases in morbidity and mortality from a number of emergent parasitic diseases. Hence changes in types and amounts of bodies of water, temperature, pH, movement, and changes in climatic condition affect prevalence and risk factor of intestinal protozoan parasite infections. Intestinal protozoa are transmitted by the fecal-oral route, water-borne and exhibit life cycles consisting of a cyst stage and a trophozoite stage. The cysts consist of a resistant wall and are excreted in the feces. The cyst wall functions to protect the organism from desiccation in the external environment. Unhygienic conditions promote transmission of most protozoa (Gascón *et al.,* 2000).The result of inter-related social, economic, cultural, historical, and political factors Control strategies involving improved drinking water supplies, excreta disposal, sewage management, sanitation, and education have been related with reduced prevalence of intestinal parasitism. Programmes of nutrition, immunization, family planning, and de-worming have been shown to effectively promote health by influencing the knowledge, perceptions, and behavior of mothers toward intestinal parasitic infections in countries (Wamani *et al.,* 2004)

2.5. Diagnosis

Intestinal protozoan parasites are widely prevalent causing considerable medical and public health problem in developing countries. Malabsorption, diarrhea, blood loss, impaired work capacity, and retarded growth can be associated with these intestinal infections and some infections occur locally in school and preschool age children (Kanmarnee *et al.,*

2004). Diagnosis of *E. histolytica* has relied on microscopic examination of protozoan morphology, but examinations by this method are unable to differentiate among protozoa with similar morphological features. A common way to distinguish *E. dispar* from *E. histolytica* microscopically is erythrophagocytosis. Classical microscopy does not allow of the invasive protozoon (*E. histolytica)* to be distinguished from the noninvasive one (*E. dispar)* unless erythrophagocytosis is seen during microscopic examination. This classical feature has long been considered the definitive diagnostic criterion for *E. histolytica.* However in some cases *E. dispar* is also observed to ingest RBCs (Haque *et al.,* 2006). Laboratory diagnosis is made by finding the characteristic cysts in iodine stained, Formol-ether concentration method or by detecting the characteristic trophozoites in a wet preparation or a permanent stained preparation. Where amebic dysentery is suspected, the laboratory should be informed that a "hot stool" is being supplied so that it can be examined within twenty minutes of being passed. On cooling the amoeba stop moving which then become very difficult to identify. Direct microscopy should be done by mixing a small amount of the specimen in 0.9% sodium chloride solution. This permits detection of motile trophozoites of *Entamoeba spp* and can also provide information on the content of the stool (i.e., the presence of leucocytes and red blood cells). On search e.g. primarily for cysts, not for Ameba, several stool samples are required to be examined, by direct microscopy and a sensitive concentration technique. Three negative stool samples are required before it can be accepted that there is no amebic infection. Microscopic examination of an amebic abscess aspirate (e.g. in the liver or lungs), may reveal hematophagous trophozoites. It must be examined immediately by mixing a drop of warm saline with some aspirated pus on a microscope slide (WHO, 2009).

Diagnosis of *Giardia* infections has been carried out using microscopic identification of cysts or trophozoites in either single or multiple stool specimens. The standard methods used to increase the sensitivity of *Giardia* detection includes iodine-stained wet smears, trichrome-stained cyst concentrates prepared by Formalin ethyl acetate centrifugation and trichrome-stained polyvinyl alcohol (PVA)-preserved stools. Much flatus trophozoites are found by examination of saline wet preparations of fresh, diarrheic stool, duodenal or jejunal aspirate or in a permanently stained fecal preparation (CDC, 2006; WHO, 2009).

Detection of *Cryptosporidium* oocysts has been performed using: histological sections of small intestine staining techniques to identify the oocysts in the feces oocyst antigen detection via immune fluorescence, enzyme linked and agglutination immuno-assays (Shrestha *et al*., 2012). Polymerase chain reaction (PCR) amplification of *Cryptosporidium* specific DNA targets Serological diagnosis of *Cryptosporidium* specific antibodies has also been applied to detect wide range of time span post infection and also can be used as a marker for epidemiological surveys (Shrestha *et al.*, 2012).

2.6. Control and Prevention of Intestinal Protozoan Parasites Infections

There are different mechanisms to prevalent intestinal protozoan parasites. To realize good preventive measures, epidemiological studies are important methods for determining the occurrence of these intestinal parasitic infections (Monib *et al*., 2016).

Prevention of intestinal protozoan parasites at present requires interruption of the fecal-oral spread of the infectious cyst stage of the parasite. Because cysts are resistant to chlorine or iodine, in developing countries water must be boiled or purified before it is safe to drink, and raw vegetables must be washed with soap and then soaked in vinegar for 15 min before they can be eaten. Since protozoan infection often spreads within a household, it is prudent to screen family members of an index case for intestinal *G.lamblia, C.parvum* and *E. histolytica* infection (Backer, 2000). Safe disposal of human and animal wastes, improved personal and environmental hygiene, proper use of latrine, early detection and treatment of drinking water and immunization pre-school and school children are the major mechanisms of prevention and control of water-borne protozoan parasitic infections (Melake *et al.,* 2003).

2.6.1. Health education

Health education and promotion of healthy behaviors can play a key role in reducing the incidence of human intestinal parasitic infections. However, the effectiveness of those activities in reducing transmission of infection varies according to different reports. In some cases, health education can decrease costs, increase levels of knowledge, and decrease re-infection rates. Health education efforts can build trust and engage communities in aspects that are crucial to the success of public health initiatives (WHO, 2011).

Health education targets encouraging personal hygiene and healthy behavior to reduce transmission and re-infection. Communities need to be educated on use of latrines, washing hands, protecting water supplies from faecal contamination, proper cooking and handling of food (Cheesebrough, 2005). Richardson *et al.*,(2012) reported that with enhanced education Programmes on sanitation and hygiene an 84% reduction in diarrhoeal diseases was realized in Bawa village in western Cameroon.

2.6.2. Improved sanitation

The most important community control measure is reduction of the source of infection through the sanitary disposal of human feces. It is important to treat all infected persons, even if they are asymptomatic, in order to reduce the possibility of contaminating the environment. The only way to completely prevent parasites from food and water is by cooking. Food prepared by individuals infected with parasites who have not thoroughly

washed their hands after using the bathroom may pose a risk. Not all water borne intestinal protozoan parasites are killed by chlorine; therefore, those organisms can exist in the water supply. Complete elimination can only be achieved by boiling for a few minutes, filtering with a one micron filter, or drinking distilled water (WHO, 2011). The principal measures that should be included in a control program consist of massive and periodic treatment of the human population to prevent environmental contamination, sanitary excreta disposal, provision of potable water and health education for the purpose of instilling personal hygiene habit in the population (Sackey *et al.*, 2003).

2.6.3. Treatments

Now a days, different groups of drugs are available that control intestinal protozoan parasites infections. Based on different age group, endemicity of the parasite and use of antimicrobial therapy vary. The most common antigiardial drug is metronidazole (CDC, 2008). Unlike other drugs, metronidazole is quickly and completely absorbed and penetrates body tissues and sections such as saliva, breast milk, semen and vaginal secretions (CDC, 2008).

3. Objective of the study

3.1. General objective

The general objective of this study was to assess amoebic and giardia infections among diarrhoeal patients in Lideta Health Center, Addis Ababa, Ethiopia.

3.2. Specific objectives

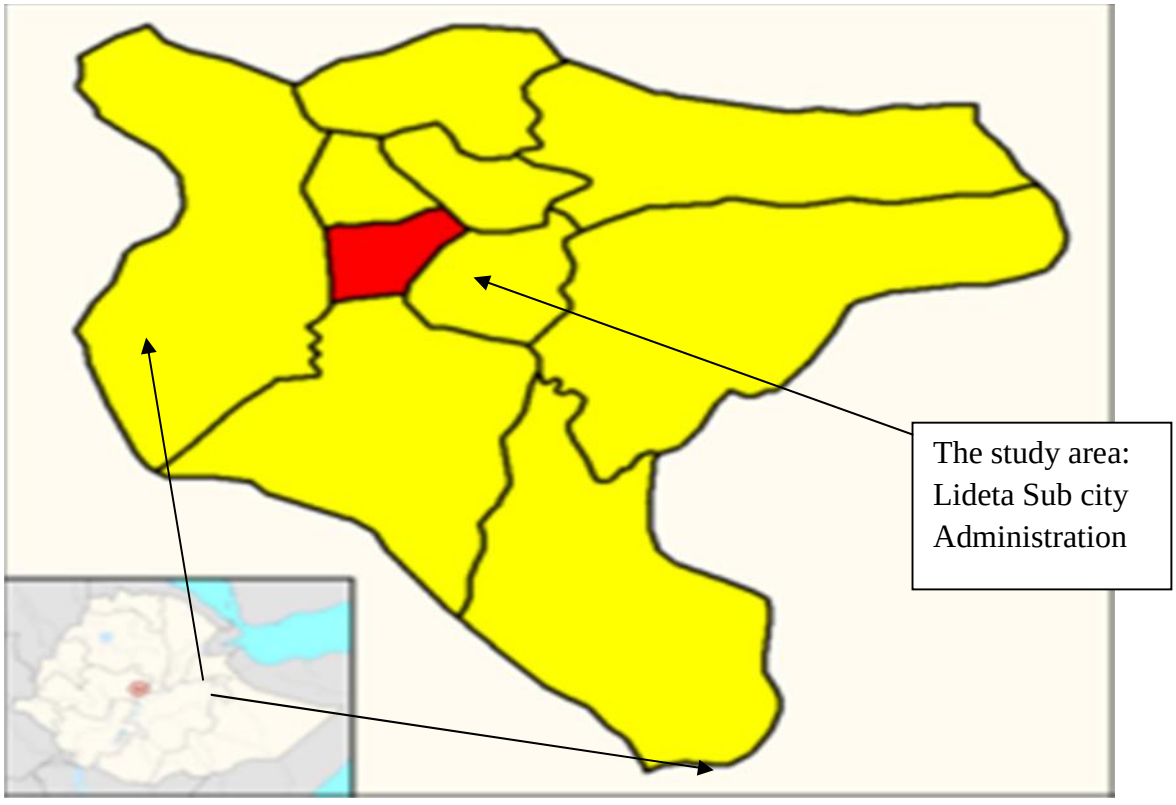
- To determine the prevalence of amoebic and giardia infections among diarrhoeal patients in Lideta Health Center.
- To identify the intestinal protozoan parasites in Lideta Health Center.
- To assess the associated risk factors for these infections in diarrhoeal patients.

4. Materials and Methods

4.1 Description of the study area

This study was conducted in woreda 10, Lideta health center which is located in Lideta Sub city Administration. The population density of this woreda is 145.11people per hector. Lideta is one of the 10 Sub cities in Addis Ababa city Administration. It is situated in the center of Addis Ababa, bounded from North by Addis Ketema Sub city, from South by Nifas Silk Lafto Sub city, from East by Kirkos Subcity and from West by Kolfe Keranyo Sub city. At present, the Sub city is divided into 10 Woredas, 27 Sub Woredas, 75 Sefers and 228 Blocks.

The total population size in the Sub city is about 201,613 and the area coverage of this sub city is 918.27 hectares or 9.18 square kilometers (CSA, 2007). Lideta is characterized by a homogenous type of topography with insignificant elevation difference. Generally speaking in the sub city, the altitude ranged from 2291 to 2424 meters above sea level which has a range of 133 meters. Most of the dwellers in the sub city are very poor with economically low income.



Source: Https://en.m.Wikipedia.org'wiki: Lideta

4.2. The Study Design

A descriptive cross-sectional survey was carried out on protozoan infections among diarrhoeal patients in Lideta sub-city health center. Laboratory examination of stool sample was carried out using direct wet mount and formol-ether concentration methods. In addition structured questionnaire was used to collect data on the patients' sex, age and socio demographic factors. The study was conducted from September 2017 to May 2018.

4.3. Determination of sample size

The sample size was determined based on the 95% confidence limits and with 5% sampling error,

$n = Z^2P\ (1\text{-}P)/d^2$

Where: n = sample required
Z = 95% confidence interval (1.96)
d = margin of error (5%)
P= prevalence rate

Since the overall infection of intestinal protozoan parasite was not known for the study area, infection would take to be 40% and the total sample size would be 240.

4.4. Stool sample collection and microscopy

Each consented patients were provided a labeled and leak proof plastic container to collect about 5g fresh stool sample. Each stool sample was microscopically examined for parasites cyst/oocyst and trophozoites. At the time of sampling; date of sampling, age, sex, presence or absence of intestinal parasitic infection and code number was recorded for each patient on record format.

4.5. Laboratory Parasitological Procedures

4.5.1. Direct Wet Mount Method

A direct wet mount with normal saline (0.85% NaCl solution) was used at study site to observe the presence of motile intestinal parasites, trophozoites under light microscope at 10X and 40X magnification. Lugol's iodine staining was also used to observe cysts of intestinal parasites (WHO, 2011).

4.5.2. Concentration Method

Using an applicator stick, approximately 2g or pea sized fecal materials was placed in a centrifuge tube containing10 ml of 10% formalin. After emulsifying the feces in the formalin, it was filtered through the nylon filter into the test tube. The filtrate was washed to discard any lumpy residue with a normal saline solution. Then after, the filtrate was washed again, by transferring into a test tube containing 7 ml of ether/ethyl acetate. The tube was closed with a stopper and it was shacked vigorously to mix. The stopper was removed and it was centrifuged at 1500 rpm for 2 minutes. The tube was rested in stand for five minutes. Four layers became visible with the top layer consisted of ether, the second was a plug of debris and the third was a clear layer of formalin and the fourth was the sediment. The plug of debris from the side of the test tube was removed with the cotton swab and poured off the liquid leaving a small amount of formalin for suspension of the sediment. Then after, the sediment was removed with a pipette. Then, a drop of fluid was added on the slide for examination under a cover slip. Some drop of iodine solution was added on the second glass slide. A 10x and 40x objectives was used to examine the whole of the deposit for ova and cysts or oocysts.

4.6. Data quality control

Questionnaires were evaluated by collecting data from patients who visited Lideta health center. The data consistency was checked at the site. All laboratory materials such as quality of reagents, sampling equipment, transporting system and microscopes were checked in Lideta health center by experienced laboratory professionals. The specimens were also checked for serial number, quality and procedures of collection. The results of the specimens were confirmed by the researcher and senior laboratory technicians in Lideta health center and it was taken as the final result of the examination.

4.7. Data analysis

The data was entered and analyzed using SPSS version 20 software. During data collection completed questionnaires were checked regularly to rectify any discrepancy, logical errors or missing values. The correlation between intestinal protozoan parasitic infection and demographic factors was computed by the regression analysis using SPSS version 20 software. The level of statistical significance was set as $p < 0.05$.

4.8. Ethical clearance

Ethical clearance was obtained from AAU College of Natural and Computational Science Institutional Review Board (CNS-IRB) Committee. The ethical considerations were addressed by referring positive individuals for treatment at the Centre. The objective of the study was explained to patients and oral consent was sought from them during stool sample collection.

5. Results

5.1. Demographic data

A total of 240 Patients were participated in the present study, of these, 122(50.8%) were males and 118(49.2%) were females (Table 1). Regarding their age 34(14.2%) were between 1-5years old, 66(27.5%) were between 6-10years old, 30(12.5%) were between 11-15years old, 35(14.6%) were between 16-20years old and 75(31.3%) were above 20years old.

Table 1: Distribution of respondents by age and sex

Variable	Category	Respondents	percentage
Gender	Male	122	50.8
	Female	118	49.2
Age (years)	1-5	34	14.2
	6-10	66	27.5
	11-15	30	12.5
	16-20	35	14.6
	>20	75	31.3

Data by gender indicated a higher number of males than females while by age, more of the patients were within the 6-10 years of age and above 20 years of age.

5.2. Prevalence of amoebic and giardia infections in diarrhoeal patients

The overall prevalence of amoebic and Giardia infections in Lideta health center among diarrheal patients was 36.3% (Table 2). Out of 240 stool samples collected 87(36.3%) were found positive for at least one intestinal protozoan species. Cysts and trophozoites of *E. histolytica* (20.8%) and *G. lamblia* (15.4%) species were found in some of the stool samples collected from patients visiting Lideta health center. The prevalence of *E. histolytica* was relatively higher among diarrhoeal patients in Lideta health center.

The prevalence of amoebic and giardia infections among age group of 1-5 was 14.3% for males and 20% for females. For the age group of 6-10 years old, 55.6% was for males and 50% was for females and for the age group of 11-15 years old, 17.6% was for male and 30.8% was for females. The prevalence of amoebic and giardia infections for the age group of 16-20 years old, 26.7% was for males and 30% was for females and for the age group of above 20 years old, 37.5% was for males and 40% for females. High prevalence

of amoebic and giardia infections was examined between the age group of 6-10 years old in both sexes (Table 2). This is certainly school age children.

Among 36.3% positive study participants, 18.1% were males and 18.2% were females. In this cross sectional study females are relatively more prevalent than males on intestinal protozoan infections (Table 2). In Ethiopia prevalence of intestinal protozoan infection of school age children in terms of gender shown variation and correlated with socio-cultural activities of the gender.

Table 2 Prevalence of IPIs among diarrhoeal patients in Lideta health center during September 2017- May, 2018.

Age group (years)	Male		Female		Both sex		P value
	Number of examined	Number of positive	Number of examined	Number of positive	Number of examined	Number of positive	
1-5	14	2(14.3%)	20	4(20%)	34	6(17.6%)	1.000
6-10	36	20(55.6%)	30	15(50%)	66	35(53.0%)	0.392
11-15	17	3(17.6%)	13	4(30.8%)	30	7(23.3%)	1.000
16-20	15	4(26.7%)	20	6(30%)	35	10(28.6%)	1.000
>20	40	15(37.5%)	35	14(40%)	75	29(38.7%)	0.489
Total	122	44(36.1%)	118	43(36.4%)	240	87(36.3%)	

In this study age was the risk factor for the prevalence of amoebic and giardia infections with no significant difference. Analysis in the study also showed that there was no significant association between male and female study participants ($p > 0.05$). 17.6% of the positive participants were between the age group of 1-5 years old. 53.0%, 23.3%, 28.6% and 38.7% were between age group of 6-10, 11-15, 16-20 and >20 years old, respectively (Table 2). The highest prevalence of amoebic and giardia infections in the study area was shown in the age groups of between 6-10 years old, indicated that these age groups were at higher risk for acquiring protozoan parasites infections. The possible reason for the highest prevalence in this age group of the present study was children have weak immune system, poor hygiene and lack of awareness to these protozoan parasites.

The studies conducted in different parts of Ethiopia indicated that 7-9 age groups have significant association with prevalence of amoebic and giardia infections. This is because children have lower resistance to amoebic and giardia infections as compared to adults. Besides, children are more exposed to overcrowded conditions in the schools and low level of awareness.

5.3. Intestinal Protozoan Species Identified from Diarrhoeal Patients

Two major species of intestinal protozoan parasites were identified from stools examination in Lideta health center which is summarized and presented in Table 3. The predominant protozoan parasites species identified in the study were *E. histolytica/dispar* (20.8%) and *G. lamblia* (15.4%). The higher prevalence of *E. histolytica/dispar and G. lamblia* infections in current study might be attributed to the fact that most children around the study area were exposed to low level of environmental sanitation, socio-economic conditions of their family, inadequate medical care and lack of awareness in simple health promotion practices such as personal hygiene and food hygiene.

Table 3: Prevalence of intestinal protozoan species identified from the study area during September 2017- May, 2018.

Age group(years) and sex	No-of examined	Intestinal protozoan species		Non-infected	P-value
		Eh/d No of pos.(%)	Gl No of pos.(%)		
1-5	34				
Male	14	-	2(14.3)	12(85.7)	1.000
Female	20	2(10)	2(10)	16(80)	
6-10	66				
Male	36	11(30.6)	7(19.4)	18(50)	0.392
Female	30	8(26.7))	9(30)	13(43.3)	
11-15	30				
Male	17	1(5.9)	2(11.8)	14(82.3)	1.000
Female	13	2(15.4)	2(15.4)	9(69.2)	
16-20	35				
Male	20	4(20)	-	16(80)	1.000
Female	15	2(13.3)	4(26.7)	9(60)	
>20	75				
Male	40	11(27.5)	6(15)	23(57.5)	0.489
Female	35	9(25.7)	3(8.6)	23(65.7)	
All age group(years)					
Male	122	27(22.1)	17(13.9)	78(63.9%)	
Female	118	23(19.5)	20(16.9)	75(63.5%)	
Total	240	50(20.8)	37(15.4)	153(63.7%)	

Eh/d = *Entamoeba histolytica/ dispar/moshoviskii*, **Gl**= *Giardia lamblia*

In the age group of 1-5 years old the prevalence of *E. histolytica/dispar* and *G. lamblia* was 2(6.7%) and 4(13.3%), respectively. In this age group the dominant parasite is *G. lamblia*. While, in the age group of 6-10 years old it was 19(28.8%) for *E. histolytica/dispar* and 16(24.2%) for *G. lamblia*. For the age group of 11-15 years old it was 3(10%) for *E. histolytica/dispar* and 4(13.3%) for *G. lamblia*. The prevalence of *E. histolytica/dispar* and *G. lamblia* for the age group of 16-20 years old was 6(17.1%) and 4(11.4%), respectively. And for the age group of above 20 years old it was 20(26.7%) for *E. histolytica/dispar* and 9(12%) for *G. lamblia*. The highest prevalence of both *E. histolytica/dispar* and *G. lamblia* was shown in age group of 6-10 years old which indicated that children in this age group are more susceptible to these intestinal protozoan infections due to weak immune systems, personal hygiene and their contact with contaminated environment.

In general the result of table 3 shown that the infection rate of *E. histolytica was* higher than the infection rate of G. *lamblia*.

5.4. Amoebic and Giardia infections with socio demographic characteristics of the respondents

This study has also analyzed correlation between socio-demographic factors and the prevalence of amoebic and giardia infections. The overall prevalence of each intestinal protozoan species diagnosed in the study of diarrheatic patients and the proportion of different socio-demographic factors are presented in (Table 4).

Among 45 participants who treated drinking water, 34(75.6%) and 11(24.4%) were found to be negative and positive for amoebic and giardia infections respectively. The remaining 195 individuals who did not treat drinking water, 44(22.6%) and 151(77.4%) were negative and positive for amoebic and Giardia infections respectively. There was statistical significance association between treatment of water and amoebic and giardia infections (p=0.024). However, there was no statistical significance association (p= 0.764) between toilet types and amoebic and giardia infections. Out of 97 participants who had public toilets, 64(65.9%) and 33(34.2%) were negative and positive for amoebic and giardia infections respectively. Whereas among 143 participants who had private toilets, 89 (62.2%) and 54(37.8%) were negative and positive for amoebic and giardia infections respectively.

In the present study, out of 84 participants who had clean toilet, 61(72.6 %) and 23(27.8%) were found to be negative and positive for amoebic and giardia infections. About156 participants of the study who had not clean toilet, 92(58.9%) and 61(41.02%) were negative and positive for amoebic and giardia infections with statistical significance difference (p = 0.042). Among 34 participants who always washed their hands before meal, 30(88.2%) and 4(11.7%) were negative and positive for amoebic and giardia

infections respectively, among 109 individuals who sometimes washed their hands, 63(57.8%) and 46(42.2%) were negative and positive for amoebic and giardia infections respectively. The remaining 97 individuals who did not wash their hands after toilet, 60(61.9%) and 37(38.1%) were negative and positive for these infections respectively. There was statistical significance association between hand washing and amoebic and giardia infections (P = 0.001).

Table 4: The correlation between independent variables and prevalence rate of amoebic and giardia infections

Parameters	Frequency	IPI		P- value
		Negative No(%)	Positive No(%)	
Treatment of water at home				
Yes	45	34(75.6)	11(24.4)	0.024
No	195	119(61.0)	76(38.9)	
Toilet type				
Public	97	64(65,9)	33(34.2)	0.764
Private	143	89(62,2)	54(37.8)	
Cleanliness of toilet				
Clean	84	61(72.6)	23(27.8)	0.042
Not clean	156	92(58.9)	64(41.02)	
Hand washing before meal or after toilet	34	30(88.2)	4(11.7)	
Always	109	63(57.8)	46(42.2)	0.001
Sometimes	97	60(61.9)	37(38.1)	
Never				
Cleanliness of kitchen utensils	29	21(75)	8(27.6)	
Very clean	87	64(73.6)	23(26.4)	0.017
Clean	124	68(54.8)	56(45.1)	
Not clean				
Eating uncooked vegetables and unwashed fruits				
Always	76	18(23.7)	58(76.3)	0.014
Sometimes	164	135(82.3)	29(16.7)	
Your meal				
Always fresh	54	47(87.3)	7(12.9)	
Sometimes fresh	69	58(84.1)	11(15.9)	0.038
Not fresh	117	48(58.9)	69(58.9)	
AIPIs				
Yes	32	23(71.8)	9(28.1)	0.048
No	208	130(62.5)	78(37.5)	

AIPIs=Awareness about intestinal protozoan infections

Among 76 participants who always ate uncooked vegetables and unwashed fruits, 18(23.7%) and 58(76.3%) were negative and positive for amoebic and giardia infections respectively whereas 164 individuals who sometimes ate uncooked vegetables and unwashed fruits, 89(54.3%) and 75(45.7%) were negative and positive for amoebic and giardia infections respectively. out of 54 participants who always ate fresh meal, 47(87.3%) and 7(12.9%) were negative and positive for amoebic and giardia infections respectively whereas 117 participants who never ate fresh meal, 48(41.01%)) and 69(58,9%) were found to be negative and positive for amoebic and giardia infections respectively. There was statistical significant association between habit of eating uncooked vegetables and eating fresh meal with amoebic and giardia infections ($p = 0.014$ and $p=0.038$) respectively.

Generally, the prevalence of amoebic and giardia infections and some risk factors such as habit of eating uncooked vegetables and unwashed fruits, hand washing before meal or after toilet, poor toilet hygiene, and individuals meal have statistical significant association. In the present study, there was high intestinal protozoan infection, but there was no statistical significant difference between some socio-demographic factors such as presence of private or public toilets and amoebic and giardia infections.

6. DISCUSSION

Different researchers have reported the prevalence rate of intestinal protozoan infections at different time in different countries and have come up with different results depending on the geographic location of the countries, surveyed, types of patients, the material and methods they used and the season they carried out the study.

Among the widely prevalent intestinal protozoan parasites in the developing countries, two were detected in the present study. The overall prevalence of this study was 36.3%, this is lower than 81.0% reported by Ashenafi and Mohammed, (2014) on Chencha town school children, but relatively nearer to 37.57% which is reported by Yimam (2016) on Mekaneselam town school age children. The prevalence of the current study was also closer to studies conducted by Ayalew (2006) in Dire Dawa villages who reported 25-45% prevalence of intestinal protozoan infection in school children. These reported differences among different studies might be associated with differences in parasitological methods used, level of environmental sanitation, drinking water source, parents' educational level and personal hygiene. High prevalence of intestinal parasitic infection is a direct manifestation of poor environmental sanitation and low level of awareness.

The most prevalent IPI in the current study was *Entamoeba histolytica/dispar*, 50(20.8%), which is relatively lower than the study conducted by (Eleni K. *et al*., 2014) with prevalence of 23.2 %. But it is higher than the study conducted by Leggesse and Erko (2004) with prevalence of 12.7% on Amoebiasis among school children in a rural area close to the South East of Lake Langanoo. These reported differences among different studies might be associated with geographical distribution of the parasites, low level of awareness, poor hygienic condition and inadequate medical care.

The second more prevalent IPI in this study was *Giardia lamblia*, 37(15.4%). This is relatively higher than the prevalence of Chencha town school children which is 11.7% as reported by (Ashenafi and Mohammed 2014) but it is nearer to 16.9% prevalence of giardiasis reported by (Eleni *et al*., 2014) on Wukro school age children. This study also comparatively higher than Mengistu *et al*.,(2007) reported with prevalence rate of 3.1%. Similarly the prevalence of giardiasis in Southwest Ethiopia was also 3.6% (Amare *et al*., 2007), this was also lower than the current study. This variation could be due to different geographical distribution of the parasites, low level of awareness, poor environmental sanitation, socio-economic conditions of the community and implementation of different prevention and control measures.

The prevalence of amoebic and giardia infections for the age group of 1-5 years old was 2(14.3%) and 4(20%) in males and females, respectively while for the age group of 6-10 years old it was 20(55.6%) for males 15(50%) for females. For the age group of 11-15 years old it was 3(17.6%) for males and 4(30.8%) for females. The prevalence of amoebic

and giardia infections for the age group of 16-20 and above 20 years old was 4(26.7%) for males, 6(30%) for female, 15(37.5%) for males and 14(40%) for females, respectively (Table 2). The higher prevalence of amoebic and giardia infections was seen in age group of 6-10 years old which indicated that lower aged children are more exposed than adults since the usually play in open fields and eat food without washing hands.

The overall prevalence of the two intestinal protozoan infections among the five age groups of the participants of the study was 6(17.6%), 35(53.0%), 7(23.3%), 10(28.6%) and 29(38.7%) for 1-5, 6-10, 11-15, 16-20 and above 20 years old, respectively (Table 2). Generally, the prevalence of amoebic and giardia infections was higher in the age group of 6-10 years old, 35(53.0%). This is because children have lower resistance to parasitic infections as compared to adults since many of the defense systems are not fully developed in children. In addition to this, children are more exposed to overcrowded conditions (schools, nurseries, playgrounds, etc).

Higher prevalence of IPI among school children may occur due to poor sanitary conditions in the schools (Oguntibeju, 2006). Children usually do not take care of their personal hygiene. For instance, they play in contaminated outdoor environments, in and around disposal sites (which can certainly cause serious health problems), face problem of absence of latrine and lack of life skills such as washing hands before and after meals (Abu Mourad, 2004).

Intestinal protozoan species identified in the stool samples of the examined diarrhoeal patients are presented in Table 3. The result of parasitological investigations showed that from 240 specimens of diarrhoeal patients, 87(36.3%) were positive for at least one intestinal protozoan parasite. Of these, 44(36.1%) and 43(36.4%) were males and females, respectively. *E. histolytica/dispar* and *Giardia lamblia* are the major protozoan parasites identified from diarrhoeal patients with prevalence of 50(20.8%) and 37(15.4%) in the study area, respectively (Table 3). In this study the prevalence of Amoebiasis caused by *E. histolytica* and giardiasis which is caused by *G. lamblia* varied in different age groups and sexes with no significance difference (Table 3).

Amoebic dysentery due to *E. histolytica* infection was the second most common cause of death in the world after malaria among intestinal protozoan parasites (WHO, 2018). The overall prevalence of the two protozoan species in the present study was 36.3%. Thus a higher prevalence of *E. histolytica/dispar* (20.8%) was found among diarrhoeal patients as compared with *G. lamblia* (15.4%).

Similar studies elsewhere have been reported from microscopic examinations. For instance, the prevalence of *E. histolytica/dispar* was reported as 8.1% in Zaria and Kaduna, Nigeria (Inabo *et al.*, 2000), 21% in Malaysia (Aza *et al*., 2003) and 11.7% in Thailand (Sirima *et al*., 2008). Another study conducted by (Ngonjo *et al*., 2012) found

the prevalence of *E. histolytica* among school children in Thika District, Kenya to be 14.6% while (Mamandou *et al*., 2010) reported an 18.8% prevalence of *E. histolytica* in school children in Njoro Disrict, Kenya.

Sex related prevalence of *G. lamblia* in patients had variation to that of *E. histolytica/dispar* where the infection showed no significant difference in the study. In the current study the prevalence of giardiasis was 17(13.9%) for males and 20(16.6%) for females while the prevalence of amoebiasis was 27(22.1%) and 23(19.5%) for males and females, respectively. On the contrary a study conducted by Macchioni *et al.,* (2015) reported a higher prevalence of giardiasis among boys than girls in Egypt. In a study conducted in Kenya, there were more males than females who contacted amoebiasis (Calderaro *et al.,* 2014). This agrees with the present study. In a countrywide survey of Amoebiasis in Ethiopia, the prevalence of amoebiasis was more among females than males in school children which show significant variations but the difference was not statistically significant among non-school communities (Mamadou *et al.,* 2010). In other study conducted in wukro town (Eleni *et al*., 2014*) E. histolytica/dispar infection* was equally distributed in both genders. Because infection by *E. histolytica/dispar* is acquired by the consumption of food or drinking water contaminated with infective cysts, the probability of infection is the same for both genders. Similarly, in studies carried out in different parts of Iran, no significant difference was observed in prevalence between males and females. The prevalence of Amoebiasis in males, 27(22.1%) was not significantly different from that in females 23(19.5%), suggesting that both the male and female individuals in the study area have the same predisposition to *E. histolytica* infection. This agrees with the report of firdu *et al.,* (2014). However, higher prevalence in female children than male children due to large sample size has been reported by Hooshar *et al.* (2004) and Sirima *et al.,* (2008).

Regarding age of the study participants, this study revealed differences in the prevalence of both Amoebiasis and giardiasis among all the five age groups but with no significant difference ($P > 0.05$). In this study higher prevalence of both Amoebiasis and giardiasis occurred in the age group of 6-10 years old indicated that this age group was at higher risk for acquiring protozoan parasites infections. The possible reason for the highest prevalence in this age group of the present study was children have weak immune system, poor hygiene, their contact with contaminated environment and lack of awareness of these protozoan parasites. Higher prevalence of protozoan parasites infections among school age children may occur due to the poor sanitary conditions in the schools and at their homes (Oguntibeju, 2006).

In endemic region, the highest infection rates have been seen in earlier age, for example in Mexico, 11% of the tested population aged 5-9 years was infected with *E. histolytica* (Caballero-Salcedo, *et al.,* 1994). This is because children have lower resistance to

amoebic and giardia infections as compared to adults that is many of the defense systems are not fully developed in children. In addition to this, children are more exposed to overcrowded conditions.

Regarding water usage practice in this study, there was a significant variation between water treatment and amoebic and giardia infections. Out of 45 participants of the study who treated drinking water, only 11(24.4%) were found to be positive while, among 195 participants who did not treat drinking water, 76(38.9%) were found to be positive for amoebic and giardia infections. This indicated that use of water that is likely to be contaminated was one of the risk factors for IPI. Water irrespective of its source can easily be contaminated during handling, especially where sanitation and personal hygiene of the individuals are generally poor. It is easy to contaminate water and food by the use of contaminated hands and utensils. A study conducted by (Atnafu, 2010) in quality water showed that 33.3 - 55.6% protozoan parasites were found in sample taken from raw surface water and public tap water. Protection of water source and treatment of water supplies have greatly reduced the parasitological and microbial load of water source (WHO, 2003). So treatment of water before consumption is very important for those people living in the study area.

In the present study, out of 84 participants who had clean toilets, only 23(27.8%) were found to be positive for amoebic and giardia infections. Among 156 participants of the study who had no clean toilets, 61(41.02%) were found to be positive for amoebic and giardia infections with statistical significant difference (P = 0.042). This was attributed to the fact that those who had no clean toilets lead to contamination of their food and water by houseflies which are agents for transmission of diseases. A study conducted by Kinuthia *et al.,* (2012) reported that most of the toilets had very low standards of cleanliness leading to many flies which occasionally settle on foodstuffs such as vegetables. However, there was no significant difference (P = 0.764) between the toilet types and prevalence of amoebic and giardia infections.

This study revealed that the prevalence of amoebic and giardia infections for those who sometimes washed their hands before meal or after toilet was 42.2% and higher than the prevalence rate of those participants who always washed their hands before meal or after toilet (11.5%) and never washed their hands after toilet (38.1%) with statistical significant difference (P = 0.001). Washing hands with soap can reduce the risk of intestinal protozoan infections by 42-47% and promotion of hand washing might safe million live per year (Curtis and Cairncross, 2003).

The prevalence of amoebic and giardia infections in regard to cleanliness of kitchen utensils was 45.1% for those study participants who had no clean kitchen utensils and higher than prevalence rate of those study participants who had very clean (27.6%) or

clean kitchen utensils (26.4%) with statistical significant difference (P = 0.017). The prevalence of amoebic and giardia infections regarding to eating uncooked vegetables and unwashed fruits (Always, Sometimes) was 58(76.3%) and 29(16.7%), respectively. Higher prevalence was observed in those study participants who ate undercooked vegetables or unwashed fruits with statistical significant difference (P = 0.014).

The prevalence of amoebic and giardia infections in relation to individuals meal always fresh, sometimes fresh, not fresh was 7(12.9%, 11(15.9%) and 69(58.9%), respectively. High prevalence was observed in those individuals who did not feed on fresh food with statistical significant difference (P = 0.038). This might due to eating unheated or over left food causes a problem on the normal functioning of the intestine and might result in diarrhoea. The prevalence of amoebic and giardia infections in relation to awareness of the study participants about environmental sanitation and personal hygiene (Yes, No) was 9(28.1%) and 78(37.5%), respectively with statistical significant difference (P = 0.048). The high occurrence of amoebic and giardia infections in the current study might be due to poor environmental sanitation (including poor disposal of sewages) which favor the persistence of cyst of the parasite in the soil and lack of awareness to these protozoan parasites. In general, significant association was observed between prevalence of human amoebic and giardia infections and habit of eating uncooked vegetables, hand washing before meal, unhygienic toilets, protection and treatment of water, , and awareness of the study participants about environmental sanitation and personal hygiene in the present study.

Environmental, socio-economic, demographic and hygiene-related behavior is known to influence the transmission and distribution of intestinal protozoan infections (Norhayati *et al.,* 2003). A study in Brazil identified place of residence, age, ingestion of raw vegetables and drinking water quality as important risk factors (Benetton *et al.,* 2005).

Prevalence of *E. histolytica* is related more too inadequate environmental sanitation and personal hygiene than to climate. Socio-economic factors as well as unpredictable factors such as food insecurity, droughts, and floods contribute to the problem (WHO, 2011). Unavailability of safe domestic water and low education on sanitation also contribute to transmission (AMREF, 2009). *E. histolytica* gains entry into the intestines through the mouth from undercooked food, vegetables, or contaminated water or hands. Poor personal hygiene, garbage disposal and poor disposal of excreta are significant for this oral-faecal infection (Blessman *et al.,* 2002). Transmission may also be through mechanical vectors such as flies (Nyarango *et al.,* 2008) whereby flies may carry the infective cysts from contaminated sites or dirty latrines and cause contamination of food or water.

Many tropical developing countries lack adequate supply of clean domestic water, contamination may occur at the source of water or at home due to poor sanitation (UNICEF,

2009). Another risk factor is the availability and usage of toilets. When people defecate in the open fields cysts of *E. histolytica* can be washed down to water bodies or may be carried by mechanical vectors such as flies and contaminate food or water sources (Cheesebrough, 2005). A study on prevalence of *E. histolytica* in a village in Côte d'Ivoire (Mamadou *et al.*, 2010) found that prevalence was high where toilets were lacking or were not used and kept clean. The same study in, Côte d'Ivoire found that the lack of toilet use despite their existence favored the spread of cysts. Another study on the risk of pathogenic intestinal parasites in Kisii municipality in Kenya associated the spread of *E. hisolytica* with poor sanitary conditions of latrines. In Vietnam, Duc *et al.*, (2009) in a study on risk factors of *E. histolytica* observed that the type of latrine used has an association to the infection rate. In their study the users of single vault latrines showed a higher prevalence.

Therefore, control measures should be taken to prevent the amoebic and giardia infections in the study area. Health extension workers should give health education encouraging personal hygiene and healthy behavior to reduce transmission and re-infection to school children in particular and to the community in general. Communities need to be educated on use of latrine, washing hands, protecting water supplies from faecal contamination, proper cooking and handling of food. Richardson *et al.*, (2012) reported that with enhanced education programs on sanitation and hygiene an 84% reduction in diarrhoeal diseases was realized in Bawa village in western Cameroon.

7. Conclusion and recommendations

7.1 Conclusion

The common intestinal parasite species diagnosed among participants of Lideta health center were *E. histolytica/dispar* and G. *lamblia*. The results of this study are an indication that *E. histolytica* and *G. lamblia* are a public health problem among the participants**.** The problem was more common in children particularly in 6-10 years old due to behavioral, social and immunological (biological) reasons.

A significant association was observed between prevalence of human amoebic and giardia infections and some risk factors such as habit of eating uncooked vegetables and unwashed fruits, unhygienic toilets, hand washing before meal or after toilet, cleanliness of kitchen utensils, and protection and treatment of water at home.

7.2. Recommendations

Providing of washed fruits and cooked vegetables, proper use and good sanitation of toilets, well protected and treated drinking water, proper education on hygienic and environmental sanitation would help in reducing the prevalence of amoebic and giardia infections and need more medical attention to avoid its consequences. More public health awareness programs should be promoted towards a better understanding of the source and adverse impacts of amoebiasis and giardiasis. Positive individuals should be treated with appropriate anti protozoan drugs. It is also recommended to local (woreda) health sector and any concerned bodies that systematic, integrated and community-participatory amoebic and giardia infections, prevention and control programs need to be implemented in the study area.

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Appendices

Appendix I - Amharic questionnaire

<u>በጎፈቃደኝነትንማረጋገጫ</u>

<u>በበጎፈቃደኞችየሚሞላመረጃመስጫናፍቃደኝነትንማረጋገጫቅፅ</u>

ጤናይስጥልኝ

በመጀመሪያ ይህን መጠይቅ ለመመለ ስፍቃደኛ ስለሆኑ ልባዊምስጋና አቀርባለሁ።።
እኔ በአዲ ስአበባ ዮኒቨርስቲ ስነህይወት ትምህርት ክፍል የድህረምረቃ ተማሪ ነኝ።የዚህ ጥናት ዋና አላማ በልደታ ጤና ጣቢያ ውስጥ ታካሚ በሆኑ በማንኛውም የእድሜ ደረጃ ላይ ያሉ እና በአንጀት ጥገኛ ተህዋሲያን የተጠቁ ሰዎችን የበሽታ አምጪ ተዋሲያን ለማጥናት ነው።።
ይህንን መጠይቅ እውነተኛና ትክክለኛ መልስ በመስጠት በታማኝነት እንድትመልሱ በትህትና ይጠይቃሉ።።የሚሰጡት መረጃ በሙሉ ሚስጥራዊነቱ የተጠበቀና ለጥናታዊ ምርምር ስራ ብቻ የሚውል ነው።።በእርስዎ ፍቃደኝነትና ስምምነት የሰገራ ናሙና ሊሰጡ ይችላሉ።።

ከላይ በተዘረዘረው ማብራሪያ መሠረት በዚህ ጥናታዊ ፅሑፍ ለመሳተፍ ከተስማሙ እባክዎ ከታች በተጠቀሰው ቅፅ ላይ ይፈርሙ።።

<u>መጠይቁን ያደረጋው</u> **<u>መረጃውን የሰጠው (ተሳታፊ)</u>**

ፊርማ ---------- ፊርማ ----------

ቀን ---------- ቀን ----------

መጠይቁን በደንብ አንብበው መልስ ብለው የመረጡትን ፊደል በማክበብ ይመልሱ።።

የጥናቱ ተሳታፊ መለያዎች

መለያ ቁጥር ----------

ወረዳ ---------- ቀበሌ ----------

ፆታ ሀ. ወንድ ለ. ሴት

እድሜ ሀ. 1-5 እድሜ ለ.6-10 እድሜ ሐ. 11-15 እድሜ
መ. 16-20 እድሜ ሠ. ከ20 እድሜ በላይ

የትምህርት ደረጃ ሀ. አንደኛ ደረጃ ያጠናቀቀ ለ. ሁለተኛ ደረጃና በላይ ያጠናቀቀ
ሐ. ያልተማረ

1. ውሃ የምት ቀዱት ከየትነው
   ሀ. ከወንዝ ለ. ከምንጭ ሐ. ከቧንቧ
   መ. ሌላከሆነይግለፁት ----------
2. በቤትዎ ውስጥ ውሃ እንዴት ይጠራቀማል?
   ሀ. በጋን ለ. በጀሪካን ሐ. በበርሜል
   መ. ሌላ ከሆነ ይግለፁት ----------

3. የውሃ ማጠራቀሚው ሁሉጊዜ ይከደናል?

ሀ. አዎ ለ. አይከደንም

4. በቤት ውስጥ የመጠጥ ውሃው ይታከማል?

ሀ. አዎ ለ. አይታከምም

መልሱ አዎ ከሆነእንዴት?

ሀ. በማፍላት ለ. ክሎሪን በመጨመር ሐ. ሌላ ከሆነ ይግለፁት -----

5. መፀዳጃ ቤት አለዎት?

ሀ. አዎ ለ. የለኝም

መልስዎ የለኝም ከሆነ የት ይፀዳዳሉ?

ሀ. ሜዳ ላይ ለ. ወንዝ ዳርቻ ሐ. በሁለቱም

6. የመፀዳጃ ቤትዎ አይነት

ሀ. የግል ለ. የሕዝብ

7. የቤት ቆሻሻዎችን በምን መንገድ ያስወግዳሉ?

ሀ. በማቃጠል ለ. ወንዝ በመጣል ሐ. ሜዳ ላይ በመጣል

መ. ሌላ ከሆ ይግለፁት ----------

8. የልጅዎ የአመጋገብ ሁኔታ

ሀ. የትምህርት ቤት ምግብ ለ. ምግብ ወደ ትምህርት ቤት በመውሰድ

ሐ. ሌላ ከሆነ ይግለፁት ----------

9. የልጅዎ ምሳ እቃ ከያዘ/ች የት ይቀመጣል?

ሀ. በመማሪያ ክፍል ውስጥ

ለ. ሌላ ከሆነ ይግለፁት ----------

10.የማብሰያ ቤትዎ ምን ይመስላል?

ሀ. በጣም ፅዱ ለ. ፅዱ ሐ. ፅዱ ያልሆነ

11.ልጅዎ ከመፀዳጃ መልስ እጆቻቸውን ይታጠባሉ?

ሀ. ሁልጊዜ ለ. አልፎአልፎ

12.ልጅዎ ያልታጠቡ አትክልትና ፍራፍሬዎችን ይመግባሉ?

ሀ. ሁልጊዜ ለ. አልፎ አልፎ

13.የመጫወቻ ቦታችሁ ለቆሻሻ ቅርብ ነው?

ሀ. አዎ ለ. አይደለም

14.የልጅዎ ምግብ

ሀ. ሁልጊዜ ትኩስ ነው ለ. አልፎ አልፎ ትኩስ ነው

15.የመፀዳጃ ቤትዎ ንፅህና

ሀ. ፅዱ ለ. በጣም ፅዱ ያልሆነ ሐ. ፅዱ ያልሆነ

16.ቀደም ብሎ ስለግልና አካባቢ ንፅህና መረጃና ስልጠና አለዎት?

ሀ. አዎ ለ. የለኝም

17.በጤና ጣቢያዎችና በጤና ኤክስቴንሽን ባለሙያዎች የሚሰጡ የጤና ትምህርት ይከታተላሉ? ሀ. ሁልጊዜ ለ. አልፎ አልፎ ሐ. አልከታተልም

Appendix II – English questionnaire

Questionnaire and Consent note

A format of gathering data for study participants

My respectful greeting to you

I would like to thank you so much for your willingness to respond this questionnaire .

I am a postgraduate student of Addis Ababa University, Addis Ababa Ethiopia. This is a postgraduate research on the topic protozoan parasitic infections among diarrheal patients in Lideta Sub-city Health Center, Addis Ababa, Ethiopia. You are asked kindly to fill the questionnaire as honest as you can. Every information you give will be treated confidentially and used for the purpose of this research work only.

b) An ensuring format for volunteer participants

On your willingness and agreement I would like to take and examine the stool from you and/or your child. If you agree to participate in the study based on the above explanation please put your signature in this format.

Thank you for your co-operation.

Investigator
Signature ----------
Date ----------

Participant
Signature _____
Date _____

Respond all the possible answer by circling the given alternative.

Study participants' identification

Code No. _____

Woreda _____ Kebele _____

Sex: a=Male b=Female

Age: a) 1-5 years b) 6-10 years
c) 11-15 years d) 16-20 years e) above 20 years

Family educational status: a) primary education b) secondary and above c) illiterate

Occupation: a) private b) government

1. Where is your main source of water?
   a) River b) spring c) tap c) other, specify ----------
2. How is water at home stored?
   a) Tanks b) jericans c) drums d) other, specify ----------
3. Is this water storage container usually covered?

a) Yes b) no

4. Do you treat drinking water at home?

a) Yes b) no

If yes how? a) Boiling b) chlorine c) other, specify ----------

5. Is there a latrine at your home?

a) Yes b) no

If no where do you dispose your stool?

a) Field b) near to river c) both

6. Type of toilet

a) Private b) public

7. How do you dispose your home garbage?

a) burning b) into river c) open ground d) other, specify ----------

8. System of your child feeding?

a) School feeding b) child carry food to school c) other, specify ----------

9. Children food storage, if they carry food from home?

a) room b) under tree c) other, specify ----------

10. Cleanliness of your kitchen utensils?

a) Very clean b) clean c) not clean

11. Do your children wash their hands after toilet?

a) Always b) sometimes c) never

12. Do your children eat unwashed fruits and vegetables?

a) Always b) sometimes

13. Is your playing area near to garbage?

a) Yes b) no

14. Your children meal is

a) Always fresh b) sometimes fresh

15. Cleanliness of your toilet is

a) Clean b) not very clean c) dirty

16. Did you get information and training about personal and environmental hygiene and sanitation before? a) Yes b) no

17. Do you attend health education from health centers and extension personnels?

a) Always b) sometimes c) never

Appendix III - Data collection format for parasitological analysis

Lab. Code No	Sex	Age	Parasite	infection	Double infections	Multiple infections	Direct Microscopy	Conc. Method	Remark

/

Appendix IV - Result report for parasitological examination

Code No. ____ Sex _____ Age _____
Address: Woreda ___ Kebele _____ Tel. _____
Type of specimen _____ Appearance _____
Date of collection _____

Ova/parasite	Positive_____	Negative _____
Parasite identified:	Direct count	Concentration count
1. _____	_____	_____
2. _____	_____	_____
3. _____	_____	_____

Others:_____
_____
_____
_____
Remark:_____
_____
_____
Date Reported_____ Signature _____

Appendix V - Laboratory data collecting format

Code	Sex	Age	Pos. neg.	date	parasites identified in stool sample		age group	infection type
				dd.mm.yr	Ehd	Gl		

Key.

Ehd= *Entamoeba histolytica/dispar* ***Pos .*= *positive,*** ***Neg.*= *negative***

Gl= *Girdia lamblia*

Declaration

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university and that all sources of materials used for the thesis have been correctly acknowledged.

Name: Abiyot Getachew

Signature ----------